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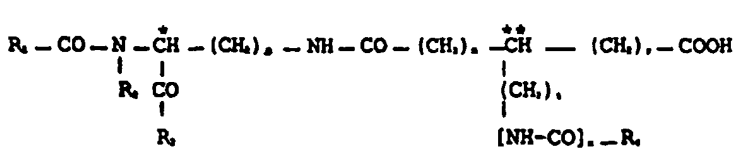
(71) Applicant *(for all designated States except US)*: ROTTA RESEARCH LABORATORIUM S.P.A. [IT/IT]; Via Valosa di Sopra, 7/9, I-20052 Monza (IT).

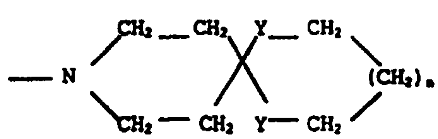
(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: MAKOVEC, Francesco [IT/IT]; Via Boito, 72, I-20052 Monza (IT). PERIS, Walter [IT/IT]; Piazza Spotorno, 3, I-20159 Milano (IT). ROVATI, Lucio, Claudio [IT/IT]; Via Ramazzotti, 1, I-20052 Monza (IT). ROVATI, Luigi, Angelo [IT/IT]; Via Valosa di Sopra, 28, I-20052 Monza (IT).

(74) Agents: RAMBELLI, Paolo et al.; Jacobacci & Perani S.p.A., Corso Regio Parco, 27, I-10152 Torino (IT).

(54) Title: POLYAMIDE DERIVATIVES OR ORNITHINE, LYSINE AND ANALOGOUS SUBSTANCES WITH CCK-B AND GASTRIN-ANTAGONISTIC ACTIVITY



(I)



(a)

(57) Abstract

Compounds are described which may be represented by general formula (I) in which: R_1 is a simple phenyl group or a phenyl grup mono- or di- substituted with chlorine; R_2 is H or CH_3; R_3 is a heterocyclic spiro ring group represented by (a) in which Y is selected independently from CH_2 and O (oxygen) and n is 0 (zero) or 1; s is a whole number from 1 to 4; m, r and t are whole numbers selected independently and ranging from 0 (zero) to 2; z is 0 (zero) or 1; R_4 is selected independently from a simple phenyl group and a phenyl group mono- or di- substituted with methyl or chlorine, a 1-(or 2-)napththyl group, a 2-(or 3-)indolyl group, a 2-(or 3-)quinolinyl group. The configuration of the chiral centre indicated * in general formula (I) may be independently D or racemic while that of the chiral centre indicated ** may be independently D, racemic or L.

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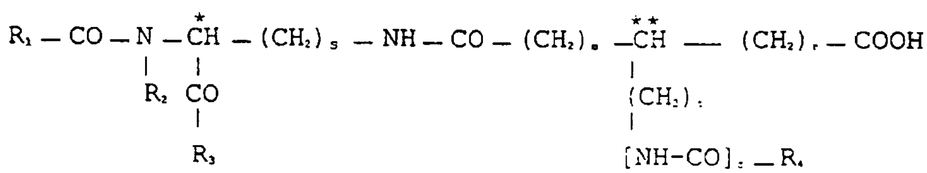
POLYAMIDE DERIVATIVES OR ORNITHINE, LYSINE AND ANALOGOUS SUBSTANCES WITH CCK-B AND GASTRIN-ANTAGONISTIC ACTIVITY

The subject of the present invention is new polyamide derivatives which may be represented by the general formula (I) shown below



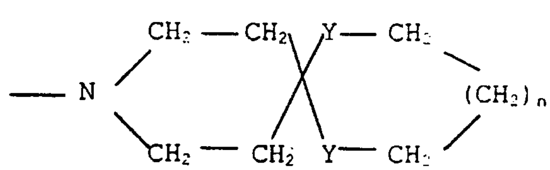
(I)

in which:

R_1 is a simple phenyl group or a phenyl group mono- or di- substituted with chlorine;

R_2 is H or CH_3;

R_3 is a heterocyclic spiro ring group represented by:



in which Y is selected independently from CH_2 and O (oxygen) and n is 0 (zero) or 1;

s is a whole number from 1 to 4;

m, r and t are whole numbers selected independently and ranging from 0 (zero) to 2;

z is 0 (zero) or 1;

R_4 is chosen independently from a simple phenyl group and a phenyl group mono- or di- substituted with methyl or chlorine, a 1-(or 2-)naphthyl group, a 2-(or 3-)indolyl group, a 2-(or 3-)quinolinyl group.

The configuration of the chiral centre indicated * in the general formula (I) may be, independently, D (dextro) or racemic (DL) while the configuration of the chiral centre indicated ** in the general formula (I) may be, independently, D (dextro), racemic (DL) or L (laevo).

Preferably R_1 is a phenyl group substituted with chlorine in the 3 and 5 positions, R_3 is the group 8-azaspiro[4.5]decan-8-yl, s is 2, R_4 is the group 1-naphthyl and, if z is 0 (zero), then m and r are both 1 while t is 0 (zero) or 1 while, when z is 1, m and t are both 0 (zero) and r is 2.

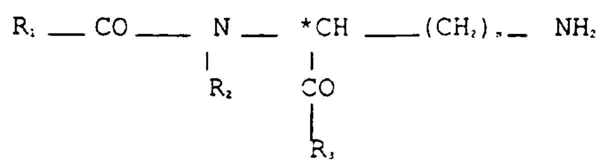
The compounds of the present invention have been shown to be powerful antagonists to the gastrin receptors at the peripheral level, that is, in the gastrointestinal tract, and powerful antagonists to the cholecystokinin (CCK) receptors at the level of the central nervous system (CCK-B-antagonists). It may thus be thought that they may be used to advantage in the treatment of various human illnesses related to imbalances in the physiological levels of gastrin and CCK or other bioactive polypeptides correlated therewith, whether in the gastrointestinal tract or in the central nervous system (CNS) or in other organs or systems in which these bioactive polypeptides play a physiological or pathological role. Thus, for example, one can foresee an advantageous use of these compounds for the treatment, at the gastrointestinal level, of illnesses connected with disorders of motility and mucous trophism such as, for example, gastritis, peptic ulcers, colitis and certain gastrointestinal tumours sustained by gastrin or polypeptide hormones correlated therewith, and at the level of the CNS, for the treatment of mental disturbances such as, for example, anxiety, panic attacks, psychosis such as, for example, schizophrenia, anorexia, etc. They may also be used in the treatment

and prevention of several pathological conditions of the eye such as, for example, myosis induced during surgical treatment for cataracts or chronic eye inflammation or other afflictions of the sensory organs.

Pharmaceutical forms of the compounds in question may be prepared by conventional techniques in the form of, for example, tablets, capsules, suspensions, solutions and suppositories, and may be administered orally, parenterally, rectal, ocularly or transdermally, or in other ways suitable for achieving the therapeutic effect.

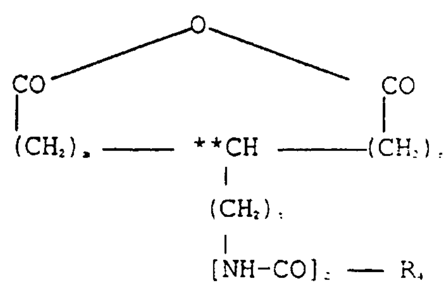
The active ingredient is typically administered to the patient in a reference dose variable from 0.01 to 10mg/kg bodyweight per dose. In the case of parenteral and ocular administration it is preferable to use a water-soluble salt of the compounds in question, such as the sodium salt or another non-toxic and pharmaceutically acceptable salt. Inactive ingredients may be substances which are commonly used in pharmaceutical methods such as eccipients, binders, aromatisers, dispersants, colorants, humectants etc.

The method for the preparation of the polyamide derivatives of the invention consists of the amidation of basic derivatives represented by the formula (II):



(II)

in which R_1, R_2, R_3, s and (*) have the meanings indicated above, with appropriate anhydrides represented by the formula (III):



(III)

in which R_4, m, r, t, z and (**) have the meanings indicated above, to give the corresponding derivatives of formula (I) in accordance with the following general reaction scheme:

$$(II) + (III) \xrightarrow{\text{Amidation}} (I)$$

Alternatively, in place of the anhydrides of formula (III) and, specifically, when m and r are both 0 (zero), the corresponding monochloride of the acid is preferably used.

The starting base derivatives of formula (II) in which the chiral centre indicated (*) is in the racemic configuration (DL) were prepared by conventional methods starting respectively from diaminopropionic acid, diaminobutyric acid, ornithine and lysine, all in the DL-configuration, to give the respective bases of formula (II) in which "s" assumes the value 1, 2, 3 and 4 (Compounds II-1 - II-4 of Table 2) respectively.

The starting base derivatives of formula (II) having the chiral centre indicated (*) in the D-configuration may conveniently be prepared from the corresponding acids, such as, for example, (D)-4-(3,5-dichlorobenzoylamino)-5-(8-azaspiro[4.5]dec-8-yl)-5-oxopentanoic acid. This compound was converted into the corresponding acyl azide which, by means of a Curtius

reaction, gave the corresponding isocyanate which, on hydrolysis, formed the desired basic compound II-5 (see Table 2). In the same way, the corresponding L-enantiomer II-6 is obtained, synthesised for the purpose of comparison. Some physico-chemical data of the basic intermediates of formula (II) used in the synthesis of the compounds of formula (I) which are the subject of the invention, are reported in Table 2. The anhydrides of formula (III) are available commercially, or they are described in the literature or were prepared by conventional methods. The following examples are given in order better to illustrate the invention.

Example 1

1-(8-azaspiro[4.5]dec-8-yl)-1-oxo-2-(DL)-(3,5-dichlorobenzoylamino)-5-aminopentane [Compound II-3 - Table 2]

7g (DL)-N^{α}-Z-N^{ε}-BOC-ornithine (0.0172 moles) prepared according to Scott J. W. et al [Synth. Comm., 1981 (11), 303-314] were dissolved in 100 mL of tetrahydrofuran (THF); 2.5 mL of triethylamine (0.0181 moles) were added to this solution which was cooled to -5°C. At this temperature 1.7 mL of ethyl chloroformate (0.0181 moles) dissolved in 20 mL of THF were added dropwise. The

reaction mixture was agitated for 20 minutes at -5°C after which, the temperature still being kept at -5°C, 2.9 g (0.0189 moles) of 8-azaspiro[4.5]decane prepared according to Najer H. et al.[Bull. Soc. Chim. Fr. 1964 (10), 2572-2581] dissolved in 30 mL of THF were added dropwise. The reaction mixture was then left for 30 minutes at -5°C and then for a night at ambient temperature. The precipitate (triethylammonium chloride) was filtered off and the THF evaporated; the oily residue was dissolved in ethyl acetate and washed first with 2N HCl and then with 2N NaOH; after washing until neutral, the solvent was dried and evaporated, 7g oil (0.0144 moles) being recovered which were dissolved in 150 mL of methyl alcohol and hydrogenated at ambient temperature and pressure in the presence of 0.2g palladium on carbon at 10% concentration. After 5 hours, the catalyst was filtered off and the solvent evaporated; 4.1g oil (0.0116 moles) were obtained which were dissolved in 100 mL of THF. 6.1 mL of 2N NaOH (0.0122 moles) and 40 mL of water were added; at 20°C, 2.5g of 3,5-dichlorobenzoyl chloride (0.0116 moles) and 6.1 mL of 2N NaOH (0.0122 moles) were added dropwise at the same time. After one night at ambient temperature the solution was acidified and the

oil which separated was extracted with ethyl acetate. The organic phase was washed with 2N HCl and water until neutral. The solvent was dried and evaporated, 6.1g of oil (0.0115 moles) being recovered which were dissolved in 100 mL of ethyl acetate; the solution was cooled to 0°C and saturated with gaseous HCl. After one hour at 0°C, the solution was treated with an excess of isopropyl ether. The precipitate was filtered, washed with isopropyl ether and dried under vacuum, giving 4.4g (0.0096 moles) of the hydrochloride of compound II-3. The yield was 56% ($C_{21}H_{29}Cl_2N_3O_2 \cdot HCl$).

Melting point 206-210°C; TLC (ButOH-Acetic acid-H_2O 5:2:2):Rf 0.75. Rotatory power; $[\alpha_D]=0$ (C=1% in chloroform).

The compounds II-1, II-2 and II-4 of Table 2 were prepared in the same way.

Example 2

1-(8-azaspiro[4.5]dec-8-yl)-1-oxo-2-(D)-(3,5-dichlorobenzoylamino)-4-aminobutane [Compound II-5 - Table 2]

5g (0.0113 moles) of (D)-4-(3,5-dichlorobenzoylamino)-5-(8-azaspiro[4.5]dec-8-yl)-5-oxo-pentanoic acid (CR 2194) were dissolved in 100 mL of dioxan; 1.58 mL of triethylamine (0.0113 moles) were added to this solution which was then cooled to -5°C. 1.08 mL of ethyl chloroformate dissolved in 25 mL of THF were added dropwise followed, after approximately 15 minutes, by 1.8 mL of trimethylsilylazide dissolved in 25 mL of THF. The temperature was then allowed to rise to ambient temperature and the procedure described by Altman Y. et al. [Tetrahedron: Asymmetry 1994 (5), p. 891, compound 9] was carried out. 2.8g (0.00678 moles) of compound II-5 were obtained. The yield was 60% ($C_{20}H_{27}Cl_2N_3O_2$).

M.p.144-147°C; TLC (ButOH-Acetic acid-H_2O 5:2:2): Rf 0.81.

Rotatory power [α_D]=-28.9° (C=1% in chloroform).

The compound II-6 of Table 2 was prepared by the same procedure, being synthesised for comparative purposes.

Example 3

4-(DL)-(1-naphthoylamino)-5N-[[3-(D)-(3,5-dichlorobenzoylamino)-4-(8-azaspiro[4.5]dec-8-yl)-4-oxo]-1-aminobutyl]-5-oxo-pentanoic acid [Compound 10 - Table 1].

7g of the compound II-5 of Table 2 (0.017 moles) were dissolved in 200 mL of methyl cyanide and 6 mL of triethylamine (0.0373 moles) were added to this solution; the mixture was cooled to -5°C and 6g (0.020 moles) of (DL)-N-(1-naphthoyl)-glutamic anhydride (compound III-5 of Table 3) were added. After 20 minutes under cold conditions the mixture was left at ambient temperature for one night. The reaction mixture was diluted with water and acidified with 2N HCl. The oil obtained in this way was extracted with ethyl acetate and subsequently washed with water until neutral, dried and evaporated. The residue obtained was precipitated by treatment with ligroin and subsequently purified by treatment with isopropyl ether. 6.5g (0.0093 moles) of the compound 10 were obtained. Yield 55% ($C_{36}H_{40}Cl_2N_4O_6$).
Mp 132-136°C; TLC (Isoamyl alcohol-Acetone-H_2O 5:2:1): Rf 0.61.

Rotatory power [α_D]=+17.8°C(C=2% in chloroform).
IR(KBr), principal peaks (cm^{-1} and % T): 3286-45%; 2939-39%; 1714-42%; 1631-15%; 804-50%; 784-50%.

All of the derivatives given in Table 1 were prepared by this method with the exception of the derivatives 3, 4

and 8 which were synthesised with the use of phenyl (compound 3) or benzylmalonic acid (compounds 4, 8) as described in the following example.

Example 4

2-(DL)-benzyl-3N-[[5-(DL)-(3,5-dichlorobenzoylamino)-6-(8-azaspiro[4.5]dec-8-yl)-6-oxo]-1-aminohexyl]-3-oxo-propionic acid [Compound 8 - Table 1].

8g (0.04 moles) of benzylmalonic acid were converted into the corresponding monochloride by treatment with thionyl chloride in a similar way to that described by Kumar G.N.et al[Drug Des. and Discovery, 1993 (10), p. 13]. The chloride, dissolved in THF, was then added dropwise at approximately 10°C to a solution containing 18g (0.04 moles) of the compound II-4 and 10.1 mL (0.08 moles) of triethylamine dissolved in 400 mL of THF. After 12 hours of reaction the solution was evaporated under vacuum and the oily residue was taken up in ethyl acetate and H_2O, the organic phase was washed with dilute HCl and water and dried under vacuum. The residue was precipitated by repeated washing with petroleum ether and then purified by treatment with isopropyl ether.

11.6g (0.0188 moles) of compound 8 were obtained. Yield 47% ($C_{32}H_{39}Cl_2N_3O_5$).

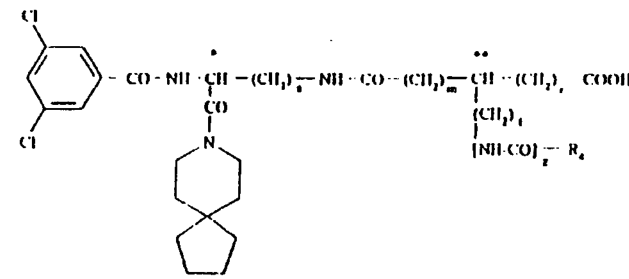
M.p.114-116°C; TLC(Isoamyl alcohol-acetone-H_2O 5:2:1):Rf 0.49.

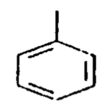
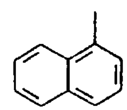
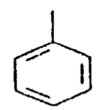
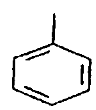
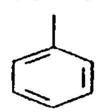
Rotatory power $[\alpha_D]$=0(C=1% in chloroform).

IR (KBr), principal peaks (cm^{-1} and % T): 3301-21%; 2939-10%;1730-24%; 1621-2%; 803-26%; 699-27%.

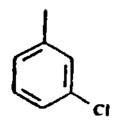
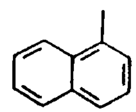
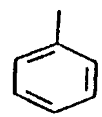
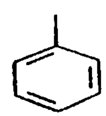
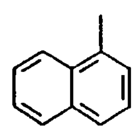
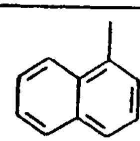
Table 1 below shows several derivatives of formula (I) obtained in this way together with some identifying physico-chemical characteristics. Table 2 shows some physico-chemical data relating to the basic intermediates of formula (II) used in the preparation of the derivatives given in Table 1, while Table 3 shows data from the literature relating to the anhydrides of formula (III) previously described together with several physico-chemical characteristics of the anhydrides (III) not previously known.

TABLE 1: COMPOUNDS OF FORMULA

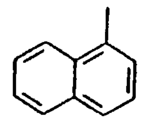
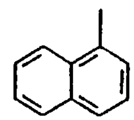
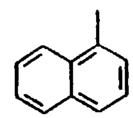
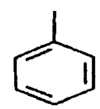
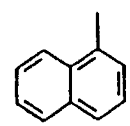
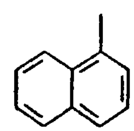


Compound	Formula	R_4	s	m	r	t	z	Configuration *	Rotatory Power (1)	Melting Point	TLC(rf) (2)
1	$C_{31}H_{37}Cl_2N_3O_5$		2	1	1	0	0	D	+30.7°	184 - 187	0.64
2	$C_{35}H_{39}Cl_2N_3O_5$		2	1	1	0	0	DL	0	105 - 116	0.59
3	$C_{30}H_{35}Cl_2N_3O_5$		3	0	0	0	0	DL	0	81 - 85	0.31
4	$C_{31}H_{37}Cl_2N_3O_5$		3	0	0	1	0	DL	0	112 - 119	0.42
5	$C_{32}H_{39}Cl_2N_3O_5$		3	1	1	0	0	DL	0	92 -96	0.56

% TABLE 1

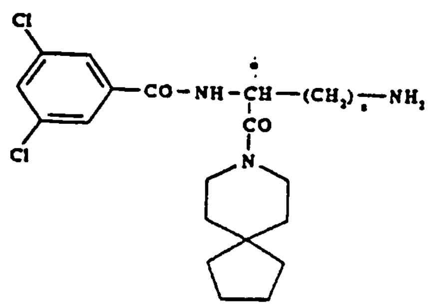
Compound	Formula	R_4	s	m	r	t	z	Configuration *	Rotatory Power (1)	Melting Point	TLC(rf) (2)
6	C32 H38 Cl3 N3 O5		3	1	1	0	0	DL	0	186 - 187	0.65
7	C36 H41 Cl2 N3 O5		3	1	1	0	0	DL	0	199 - 202	0.58
8	C32 H39 Cl2 N3 O5		4	0	0	1	0	DL	0	114 - 116	0.49
9	C33 H41 Cl2 N3 O5		4	1	1	0	0	DL	0	89 - 91	0.8
10	C36 H40 Cl2 N4 O6		2	0	2	0	1	D	+17.8°	132 - 136	0.61
11	C36 H40 Cl2 N4 O6		2	2	0	0	1	D	+23°	131 - 138	0.33

% TABLE 1

Compound	Formula	R_4	s	m	r	t	z	Configuration *	Rotatory Power (1)	Melting Point	TLC(rf) (2)
12	C37 H42 C12 N4 O6		4	0	1	0	1	DL	0	183 - 185	0.58
13	C38 H44 C12 N4 O6		4	0	2	0	1	DL	0	174 - 182	0.58
14	C38 H44 C12 N4 O6		4	2	0	0	1	DL	0	115 - 121	0.35
15	C32 H39 C12 N3 O5		2	1	1	1	0	D	+28.4°	73 - 75	0.67
16	C34 H37 C12 N3 O5		1	1	1	0	0	DL	0	151 - 157	0.7
17	C35 H39 C12 N3 O5		2	1	1	0	0	L	-38.7°	125 - 127	0.6

In compounds 1-17 the configuration of the chiral centre indicated ** is always (D,L).
(1): c = 2 g/100 mL in chloroform.
(2): eluent isoamyl alcohol/acetone/water 5:2:1 (v/v/v).

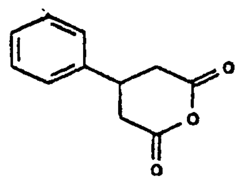
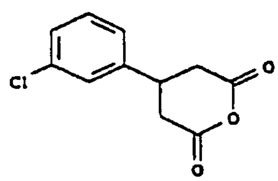
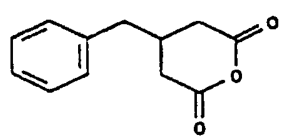
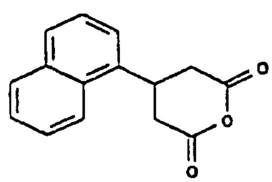
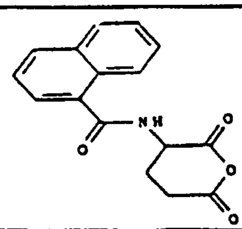
TABLE 2; COMPOUNDS OF FORMULA



Compound	Formula	s	Configuration *	Rotatory Power (1)	Melting Point (2)
II-1	C19 H25 C12 N3 O2	1	DL	0	163 - 165
II-2	C20 H27 C12 N3 O2	2	DL	0	>260
II-3	C21 H29 C12 N3 O2	3	DL	0	206 - 210
II-4	C22 H31 C12 N3 O2	4	DL	0	132 - 135
II-5	C20 H27 C12 N3 O2	2	D	-28.9°	144 - 147
II-6	C20 H27 C12 N3 O2	2	L	+27.8°	141 - 148

(1): c = 1 g/100 mL in chloroform.
(2): the compounds II-2, II-3 and II-4 were isolated as hydrochlorides.

TABLE 3; ANHYDRIDES OF FORMULA (III)

Compound	Structure	Formula	Melting Point	CAS-RN
III-1		C11 H10 O3	--	4160-80-9
III-2		C11 H9 Cl O3	--	4759-62-0
III-3		C12 H12 O3	--	91963-19-8
III-4(*)		C15 H12 O3	168 - 170	--
III-5(*)		C16 H13 N O4	187 - 189	--

(*): the anhydrides III-4 and III-5 were synthesized according to the method described by Makovec et al. [European Pat. Appl. 87830442.7].

Description of the pharmacological activity

1) Anti-cholecystokinin activity (anti-CCK-B) in vitro

In order to evaluate the capacity of the compounds which are the subject of the invention to interact with the central CCK-B receptor, [3-H][N-methyl-N-leucine]CCK-8 was used. Binding was shown to be selective for the CCK-B receptors, having an affinity approximately 4000 times greater for the cortical receptors (CCK-B) than for those in the pancreas (CCK-A) in guinea pigs [Knapp et al;J.Pharmacol. and Exp.Therap. 255 (3)(1990), 1278-1286].

The cerebral cortices of male albino guinea pigs were therefore used, the method described above being followed with slight modifications [Makovec et al.;Bioorganic & Med. Chem. Letters 3 (5)(1993), 861-866] and in such a way as to obtain a membrane content corresponding to approximately 300 mcg of protein/ml. The results obtained are shown in Table 4 which gives the IC_{50}, that is, the concentration (in moles/litre) of the antagonist capable of displacing 50% of the [3-H][N-methyl-N-leucine]CCK-8 from the receptor.

TABLE 4: Inhibition of binding of (^{3}H)-[N-Methyl-N-Leucine]-CCK-8 to the cortical membranes in guinea pigs

Compound	IC_{50} x 10^{-8} M	Compound	IC_{50} x 10^{-8} M
1	5.7	11	7.7
2	2.5	12	52.8
3	IN (>100)	13	19.8
4	25.7	14	40.3
5	16.3	15	7.6
6	13.9	16	62.6
7	9.1	17	76.7
8	27.7	CR 2194	240
9	67.3	Pentagastrin	0.3
10	1.2		

From the data reported in Table 4 it can be seen that many of the compounds in question, such as, for example, compounds 2 and 10, are powerful inhibitors of the binding of [N-methyl-N-leucine]CCK-8 to the cortical membrane receptors in guinea pigs, being only 4-8 times less active than the specific antagonist, pentagastrin, and 100-200 times more powerful than the reference compound CR 2194. The displacement activity is strongly influenced by the stereochemistry of the carbon atom indicated (*) in the general formula (I). In fact, for example, the compound 2 is approximately 30 times more active than its L-diastereoisomer, the compound 17.

2) <u>Antigastrin activity (peripheral) in rabbit gastric mucosa cells in vitro</u>

The parietal cells of the gastric mucosa are responsible for the secretion of HCl. They have specific membrane receptors which may be activated by gastrin and which have been defined as gastrin or type-B cholecystokinin receptors (CCK-B).

Since it has been observed that activation of the CCK-B receptors by gastrin leads to an increase in the level of

cytosolic calcium ions, a technique was used for measuring the increase in intracellular calcium caused by gastrin in the presence and in the absence of the compounds in question to give an indication of the antigastrin activities of the compounds themselves.

Suspensions (0.8×10^6/ml) of rabbit gastric mucosa cells were prepared by conventional techniques with the use of collagenase and pronase as digestive enzymes; estimation of the levels of $[Ca^{2+}]_i$, basal or obtained after stimulation of the cellular system, were conducted in accordance with Grynkiewicz et al[J.Biol.Chem. 260 (1985), 3440]. In the control samples the cells were stimulated with 5×10^{-8} gastrin while, in the samples in which the effect of the subject compounds was evaluated, the cells were incubated therewith before being stimulated with gastrin. The results were expressed as percentage $[Ca^{2+}]_i$ increases with respect to the control value. The anti-gastrin activity of the compounds was expressed as IC_{50}, that is, the concentration (in mmoles/litre) at which the response to the stimulus caused by gastrin is reduced by 50%. The results obtained in this way for several of the compounds in question are reported in Table 5, which also gives an index obtained from the ratio between the peripheral

anti-gastrin activity just described and the displacement activity derived from the study of binding to the cortical receptors in guinea pigs as previously described.

TABLE 5: Inhibition of the increase in cytosolic calcium caused by gastrin in rabbit gastric mucosa cells

Compound	$IC_{50} \times 10^{-8}$ M	Ratio $\frac{IC_{50}\text{ Binding cortex}}{IC_{50}\text{ (Gastric Mucosa)}}$ *
1	3.0	1.9
2	0.5	5.0
3	IN (>100)	----
4	3.5	7.3
5	8.2	2.0
6	3.0	4.6
7	1.9	4.8
8	15.4	1.8
9	20.5	3.3
10	0.3	4.0
11	1.5	5.1
12	6.6	8.0
13	4.8	4.1
14	22.8	1.8
15	3.2	2.4
16	17.7	3.5
17	IN (>100)	----

(*) values taken from Table 4

From the data given in Table 5, it can be seen that many of the compounds in question are powerful inhibitors of the increase in cytosolic calcium caused by gastrin in rabbit gastric mucosa cells.

The peripheral anti-gastrin activity essentially accords well with the anti-gastrin activity obtained centrally in the binding studies illustrated previously in Table 4. In fact, the compounds 2 and 10 are again the most powerful of the compounds described, exhibiting an IC_{50} of nanomolar order of magnitude (5 and 3 nM respectively). Generally, the compounds in question have an anti-gastrin activity on this model at concentrations 2-8 times less than those obtained centrally.

3) Anti-cholecystokinin activity (anti-CCK-A)

In order to verify the hypothesis that the compounds in question are specific CCK-B antagonists, all of the compounds illustrated in Tables 4 and 5 were tested for possible CCK-A antagonist activity. As an experimental model, the guinea pig gall bladder was used which was stimulated by CCK-8 in vitro according to the method

described by Makovec et al[(Arzneim. Forsch / Drug. Res. 35 (7), 1048-1051 (1985)].

None of the compounds tested demonstrated a CCK-A antagonist activity more powerful than 10 x 10^{-6} M.

A comparision of these activities with the CCK-B antagonist activities illustrated previously in Table 5, leads to the conclusion that the compounds in question are specific antagonists for the CCK-B receptor, the more powerful compounds, such as the compounds 2 and 10, showing an affinity at least 1000 times greater for the gastrin receptor (CCK-B) than for the cholecystokinin receptor (CCK-A).

4) Anxiolytic activity

Among the possible therapeutic activities of the components in question on the central nervous system related to imbalances in the neural physiological levels of gastrin or other peptides correlated therewith, their potential anxiolytic activity appears particularly interesting.

In fact, an important role for the central CCK-B receptor in anxiety has recently been postulated. This accords with studies also conducted in humans which have demonstrated that the central CCK-B mechanisms have an important function in the mediation of panic attacks [Bradwejn, J. et al; J.Psychopharmacology 6 (1992), 345]. In order to confirm this hypothesis, the anxiolytic activities of several of the most powerful CCK-B antagonists which are the subject of the invention were evaluated with the use of the "elevated plus-maze" method in rats, conducted in accordance with Pellow et al. [J.of Neurosc. Meth. 14 (1985), 149-167]. A labryinth was used in which the length of the cross arm was 45cm, the arm being positioned 70cm above the ground. In this experimental model, a compound having anxiolytic activity produces a percentage increase in the time spent in the open arms and a percentage increase in the number of entries into the open arms.

The results obtained are shown in Table 6 below, which shows the activities obtained with various doses of the compound 10 administered intraperitoneally (IP) in comparision with a group of animals treated with a physiological saline administered via the same route.

TABLE 6: Anxiolytic activity in the rat using the "Plus Maze" test.

Compounds	Dose mg/kg IP	N$_o$. Animals	Open Arm Entries%/Total	% Effect Vs controls	Open Arm Time/ Total time (%)	% Effect Vs controls
Controls	--	12	20.9	--	10.1	--
Compound 10	0.01	12	25.9	24.2	14.7	45.8
"	0.1	12	30.9(*)	48.3	23.1(*)	128.2
"	1.0	12	34.0(*)	63.0	24.5(*)	142.3

(*): Duncan test: $p<0.05$ vs control group

An examination of Table 6 reveals that the compound 10 exhibits a powerful anxiolytic effect.

One can, in fact, see that at doses of 0.1 and 1.0mg/kg IP the compound increases the percentage of entries into the open arms over the number of total entries by approximately 50-60% and in a significant way when compared with the controls.

In addition, the compound 10, increases the percentage time spent in the open arms at all doses; this increase is significant for doses of 0.1 and 1mg/kg IP compared with the group of control animals treated with physiological saline only.

CLAIMS

1. Compounds which may be represented by the general formula (I) indicated below

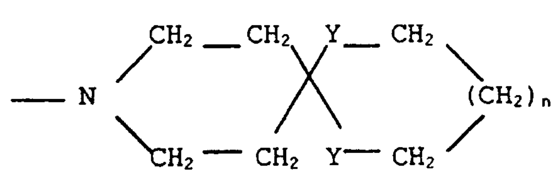
$$
\begin{array}{l}
R_1 - CO - N - \overset{*}{C}H - (CH_2)_s - NH - CO - (CH_2)_m - \overset{**}{C}H - (CH_2)_r - COOH \\
\qquad\qquad\quad | \quad\;\; | \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad\;\; | \\
\qquad\qquad\; R_2 \;\; CO \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\; (CH_2)_t \\
\qquad\qquad\qquad\;\; | \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\; | \\
\qquad\qquad\qquad R_3 \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\; [NH-CO]_z - R_4
\end{array}
\qquad (I)
$$

in which:

R_1 is a simple phenyl group or a phenyl group mono- or di- substituted with chlorine;

R_2 is H or CH_3;

R_3 is a heterocyclic spiro ring group represented by:



in which Y is chosen independently from CH_2 and O (oxygen) and n is 0 (zero) or 1;

s is a whole number from 1 to 4;

m, r and t are whole numbers chosen independently from 0 (zero) to 2;

z is 0 (zero) or 1;

R_4 is chosen independently from a simple phenyl group or a phenyl group mono- or di- substituted with methyl or chlorine, a 1-(or 2-)naphthyl group, a 2-(or 3-)indolyl group, a 2-(or 3-)quinolinyl group;

the configuration of the chiral centre indicated * in the general formula (I) may be, independently, D (dextro) or racemic (DL), while the configuration of the chiral centre indicated ** in the general formula (I) may be, independently, D (dextro), racemic (DL) or L (laevo); preferably R_1 is a phenyl group substituted with chlorine in the 3 and 5 positions, R_3 is the 8-azaspiro[4.5]decan-8-yl group, s is 2, R_4 is the 1-naphthyl group and, if z is 0 (zero), at the same time m and r are both 1 while t is 0 (zero) or 1, while, if z is 1, m and t are both 0 (zero) and r is 2.

2. Compounds according to Claim 1 of general formula (I) in which R_1 is the 3,5-dichlorophenyl group, R_2 is H or CH_3, R_3 is the 8-azaspiro[4.5]decan-8-yl group, R_4 is the 1-naphthyl group, s is 2 or 3, m, r, t, are independently 0 (zero) or 1, z is 0 (zero) and the

stereochemistry of the chiral centre indicated (*) in (I) is D (dextro) or (DL).

3. Compounds according to Claim 1 of general formula (I) in which R_1 is the 3,5-dichlorophenyl group, R_2 is H or CH_3, R_3 is 8-azaspiro[4.5]decan-8-yl group, R_4 is the 1-naphthyl group, s is 2 or 3, m and t are 0 (zero), r is 2, z is 1 and the stereochemistry of the chiral centre indicated (*) in (I) is D (dextro) or (DL).

4. A pharmaceutical preparation including at least one of the compounds according to Claim 1 or a pharmaceutically acceptable salt thereof as its active ingredient.

5. A pharmaceutical preparation according to Claim 4 for therapeutic use as a function of its anti-ulcer activity.

6. A pharmaceutical preparation according to Claim 4 for therapeutic use against tumours sustained by gastrin and other bioactive polypeptides correlated therewith.

7. A pharmaceutical preparation according to Claim 4 for therapeutic use in disorders of the gastrointestinal tract such as non-ulcerous dispepsia and irritable colon.

8. A pharmaceutical preparation according to Claim 4 for the treatment of pathological conditions of the CNS elated to imbalances in the neural physiological levels of gastrin or other bioactive polypeptides correlated therewith, such as, for example, anxiety, panic attacks, psychosis, anorexia etc or other causes related to the mechanism of the action of the compounds according to Claim 1.

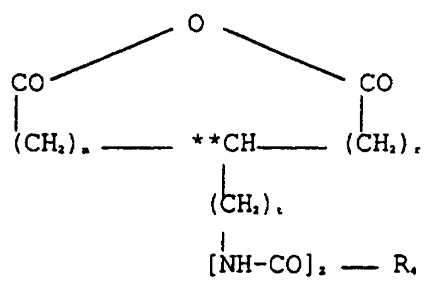
9. A pharmaceutical preparation according to Claim 4 for use in the treatment and prevention of pathological conditions of the eye such as myosis caused by the surgical treatment of cataracts, or chronic occular inflammation, or for the prevention of diseases of other sensory organs related to the mechanism of the action of the compounds according to Claim 1.

10. A pharmaceutical preparation according to Claim 4 further including, in addition, pharmaceutically acceptable inactive ingredients chosen from the group which comprises vehicles, binders, aromatisers, dispersants, preservatives, humectants, and mixtures thereof, or ingredients which facilitate transdermal absorption.

11. A method for the preparation of a derivative according to the general formula (I) in which R_1, R_2, R_3, R_4, s, m, z, r, t have the meanings indicated in Claim 1 and in which the substituents on the chiral centre indicated (*) have the D- or (DL) configuration and the substituents on the chiral centre indicated (**) have the D-, (DL) or L- configuration, which consists of the amidation of the basic derivatives represented by the formula (II):

$$\begin{array}{ccccccccc} R_1 & - & CO & - & N & - & {}^{*}CH & - & (CH_2)_s - NH_2 \\ & & & & | & & | & & \\ & & & & R_2 & & CO & & \\ & & & & & & | & & \\ & & & & & & R_3 & & \end{array} \qquad (II)$$

in which R_1, R_2, R_3, s and (*) have the meanings indicated above, with appropriate anhydrides represented by the formula (III):



(III)

in which R_4, m, r, t, z and (**) have the meanings indicated previously, in a molar ratio of from 1 to 3, in the presence or absence of a tertiary base and at a temperature of between -15°C and +20°C, or by other equivalent conventional methods of synthesis with the use, for example, of the monochlorides of the appropriate dicarboxylic acids in the same manner of procedure as indicated above for the corresponding anhydrides, the recovery of the compounds (I) as such from the reaction mass or as pharmaceutically acceptable salts thereof and their purification by conventional methods.